



(AK)

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>5</sup> :</b>  <b>G01N 33/543</b>	<b>A1</b>	<b>(43) International Publication Number:</b> <b>WO 94/09366</b>  <b>(43) International Publication Date:</b> <b>28 April 1994 (28.04.94)</b>
<b>(21) International Application Number:</b> PCT/US93/08751 <b>(22) International Filing Date:</b> 16 September 1993 (16.09.93) <b>(30) Priority data:</b> 958,068 8 October 1992 (08.10.92) US <b>(71) Applicant:</b> ABBOTT LABORATORIES [US/US]; CHAD-0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US). <b>(72) Inventors:</b> FORNEY, Kevin, J. ; 7206 3rd Avenue, Kenosha, WI 53143 (US). GEIST, Jill, M. ; 35914 N. Ash Street, Ingleside, IL 60041 (US). LOOMIS, Neil, W. ; 1730 S. Wisconsin Avenue, Racine, WI 53403 (US). MORICI, Laura, S. ; 813 Burr Oak Circle, Cary, IL 60013 (US). MUETTERTIES, Andrew, J. ; 708 Country-side Highway, Mundelein, IL 60060 (US). PARSONS, Robert, G. ; 1621 Wickham Court, Green Oaks, IL 60048 (US). PUTMAN, Jill, M. ; 210 Yvonne Court, Round Lake Beach, IL 60073 (US). ROPELLA, Paul, J. ; 3485 Oak Tree Lane, Racine, WI 53405 (US). SCHAPIRA, Thomas, G. ; 21616 107th Street, Bristol, WI 53104 (US). SIEGEL, Neal, A. ; 334 Willow Avenue, Deerfield, IL 60015 (US). WAGNER, Brian, K. ; 734 N. 22nd Street, #5, Milwaukee, WI 53233 (US). BASORE, Bob, O. ; 704 Shootershill, Toledo, OH 43617 (US).		<b>(74) Agents:</b> GORMAN, Edward, H., Jr. et al.; Abbott Laboratories, CHAD-0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US).  <b>(81) Designated States:</b> AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> ASSAY DEVICES USING SUBSURFACE FLOW  <b>(57) Abstract</b>  The present invention is directed to improving the performance of assays using a disposable assay device which includes a porous material in liquid communication with a capillary track. In particular, the capillary track is used in conjunction with the solid support to direct test sample and assay reagents directly to a defined reaction site on or in the porous material. Signal development at the reaction site indicates the assay result. The present invention is also directed to the construction of a disposable assay device which includes a capillary track. In particular, the capillary track is formed by printing a fluid insoluble material, in the reverse image of the desired capillary track, on a film layer and then capping the printed material. Alternatively, the capillary track is formed by printing a fluid insoluble material, in the reverse image of the desired capillary track, on a porous material which is then sandwiched between two film layers.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

## ASSAY DEVICES USING SUBSURFACE FLOW

## BACKGROUND OF THE INVENTION

5 1. Field of the Invention

In general, the present invention relates to assay methods and devices for the detection of an analyte in a test sample. In particular, the invention relates to novel test devices designed to provide for the rapid transfer of fluid to a reactive membrane by means of a capillary track. In addition, the invention relates to novel  
10 methods for forming a capillary track.

2. Related Art

Various analytical procedures and devices are commonly employed in assays to determine the presence and/or amount of substances of interest which may be  
15 present in biological or non-biological fluids. Such substances are commonly termed "analytes". The ability to use materials which specifically bind to an analyte of interest has created a burgeoning diagnostic device market based on the use of binding assays.

Binding assays incorporate specific binding members, typified by antibody  
20 and antigen immunoreactants, wherein one member of the specific binding pair is labeled with a signal-producing compound. For example, in a binding assay the test sample suspected of containing analyte can be mixed with a labeled anti-analyte antibody, i.e., labeled reagent, and incubated for a period of time sufficient for the immunoreaction to occur. The reaction mixture is subsequently analyzed to detect  
25 either that label which is associated with an analyte/labeled reagent complex (bound labeled reagent) or that label which is not complexed with analyte (free labeled reagent). As a result, the presence or amount of label in one of these species can be correlated to the presence or amount of analyte in the test sample.

The solid phase assay format is a commonly used binding assay technique.  
30 There are a number of assay devices and procedures wherein the presence of an analyte is indicated by the binding of the analyte to an labeled reagent and/or an immobilized complementary binding member. The immobilized binding member is bound, or becomes bound during the assay, to a solid phase such as a dipstick, teststrip, flow-through pad, paper, fiber matrix or other suitable solid phase  
35 material. The binding reaction between the analyte and/or assay reagent(s) results in a distribution of the labeled reagent between that which is immobilized upon the

solid phase and that which remains free. The presence or amount of analyte in the test sample is typically indicated by the extent to which the labeled reagent becomes immobilized upon the solid phase material.

Flow-through pads for immobilizing and detecting an analyte are well-known in the art. For example, Tom et al. (United States Patent No. 4,366,241) disclose a  
5 bibulous strip with an immunosorbing zone to which the test sample is directly applied and wherein the assay result is detected.

The use of reagent-impregnated teststrips in specific binding assays is also well-known. In such procedures, a test sample is applied to one portion of the  
10 teststrip and is allowed to migrate or wick through the strip. Thus, the analyte to be detected or measured passes through or along the strip, possibly with the aid of an eluting solvent which can be the test sample itself or a separately added solution. The analyte migrates into a capture or detection zone on the teststrip, wherein a complementary binding member to the analyte or labeled reagent has been  
15 immobilized. The extent to which the analyte becomes bound in the detection zone can be determined with the aid of the labeled reagent which can also be incorporated in the teststrip or which can be applied separately.

An early teststrip device is described by Deutsch et al. in United States Patent No. 4,361,537. In general, the device comprises a material capable of transporting  
20 a solution by capillary action, i.e., a wicking or chromatographic action. Different areas or zones in the teststrip contain the assay reagents needed to produce a detectable signal as the analyte is transported to or through such zones. The device is suited for both chemical assays and binding assays and uses a developer solution to transport analyte along the strip.

25 The disadvantages of the conventional porous or absorbent matrix devices include the slow rate of flow of test sample through the teststrip material. In addition, the test sample and mobile reagents are directed to and through the edge of an absorbent pad or layer containing the reaction site. Such diffuse sample application results in reduced signal production and a slowed rate of signal  
30 production.

Assay devices have also been constructed of tubes wherein the capillary tube contains an immobilized assay reagent to define a reaction zone for the capture and detection of an analyte of interest (Hibino et al., 4,690,907). In general, the capillary tube is used to collect a predetermined amount of test sample for use in a  
35 test device.

Conventional capillary tracks are formed from glass tubes. Glass tubes, however, are usually restricted to simple geometric designs. Glass tracks are also breakable and a biosafety hazard to workers. Other typical capillary tracks are constructed by sandwiching a die-cut material between two pieces of film, wherein one film is typically more hydrophobic than the other for the purpose of promoting fluid movement. This type of device is limited to simple single track designs because of manufacturing limitations involving the placement of the die-cut middle layer.

In still another conventional design, capillary tracks are formed through a process of injection molding. A major disadvantage of this process is the cost of prototyping. Another disadvantage is in the manufacturing process which is limited to piece-part assembly. Also, the use of multiple materials complicates construction and assembly. When dissimilar materials are incorporated as different layers, the separate pieces must be spot treated during assembly. For example, a sandwich layer of adhesive would be needed to mate the pieces together. Alternatively, the materials could be ultrasonically welded or solvent bonded, but the manufacturing limitations remain.

#### SUMMARY OF THE INVENTION

The present invention is directed to improving the performance of assays using a disposable assay device which includes a porous material in liquid communication with a capillary track. In particular, the capillary track is used in conjunction with the solid support to direct test sample and assay reagents directly to a defined reaction site on or in the porous material. Signal development at the reaction site indicates the assay result.

The present invention is also well suited for enhancing the production of signal at the reaction site. The capillary track directs fluid to a position below the defined reaction site on the porous material such that the fluid does not have to pass through the edge of the porous material, as in conventional teststrip device, to reach the reaction site. Preferably, the test sample is directed to a position directly below the reaction site on the porous material. Upon contact with the porous material, the fluid passes radially through the reaction site rather than transversely through the site.

The devices of the present invention are constructed from a capillary track having an inlet and outlet, wherein the inlet receives test sample or test solution, and the outlet is in communication with and directs test sample or test solution to a

porous support containing an immobilized reagent which binds to the analyte, an ancillary specific binding member or a labeled reagent to produce a detectable assay result. The outlet port is disposed beneath the immobilized reagent in the porous support. The device may further include a labeled reagent such that the reagent need  
5 not be separately contacted to the device and such that the assay method is self-performing. The labeled reagent may be contained within the capillary track or within a material or means which is in fluid communication with the capillary track. In a preferred embodiment, a reagent matrix is contained within a drop forming means which is in communication with the capillary track. Assay kits are also  
10 contemplated and include the subsurface flow device together with one or more containers of reagents necessary to the performance of the assay.

The present invention is also directed to constructing a disposable assay device which includes a capillary track. One method for constructing the capillary track involves applying a printable material to a first film layer thereby forming a  
15 core layer and three sides of the capillary track, wherein the printable material is deposited as a reverse image of the capillary track on the first film layer. A second film layer is then adhered to the top of the printable material or core layer, thereby forming the fourth side of the capillary track.

An alternative method for constructing the capillary track involves applying  
20 a fluid repellant printable substance to a length of porous material thereby impregnating the porous material wherein a non-impregnated region defines two sides of the capillary track. A first and a second film layer are then adhered to the top and bottom of the porous material thereby forming the top and bottom of the capillary track.

25 The present invention can also be adapted for use in the automated diagnosis of a plurality of samples. Another object of the present invention is to provide a device capable of performing multiple, highly sensitive, diagnostic tests simultaneously on a single sample in a single device having multiple capillary tracks and reaction sites. In particular, the devices of the present invention can be used in an automated  
30 fashion where the assay reaction can be rapidly performed and monitored with a minimum of sample material.

#### BRIEF DESCRIPTION OF THE DRAWINGS

35 Figure 1 is a side perspective of one embodiment of the present reaction device.

Figure 2 is a side perspective of the present reaction device showing two layer construction.

Figure 3 is a side perspective of the present reaction device showing three layer construction. Figure 3a depicts a modified embodiment of the present invention.

Figure 4 is an end view of a reaction device showing a printed layer construction.

Figure 5 is an end view of a reaction device showing a multi-layer construction using a partially impregnated porous material as the middle layer.

Figure 6 is an end view of a reaction device showing a three layer construction.

Figure 7 is a top perspective of an enhanced assay device having directed flow through the reaction site.

Figure 8 is side perspective of an enhanced assay device having an absorbent layer.

Figure 9 depicts predicted chromatographic flow rates in a linear and radial flow format.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20

One exemplary embodiment of the present invention is shown in Figure 1. The device (10) includes a main body (12) in which a capillary track (18) extends along at least a portion of its length. The capillary track has a size and dimensions suitable for the transport of test sample through the track by capillary action. The capillary track has an inlet (14) for the introduction of test sample to the device. The capillary track is in fluid communication with a porous support (20) containing an immobilized specific binding material (22). The capillary track has an outlet (16) in fluid flow communication with the porous support. Preferably, the porous support is positioned such that the outlet of the capillary track lies directly beneath the site of the immobilized specific binding material. For purposes of the present invention, "directly beneath" means that fluid passing from the capillary track to the porous support passes through less than one-half the largest dimension of the porous support. More preferably, fluid passing from the capillary track to the porous support passes through less than one-quarter the largest dimension of the porous support.

Before proceeding with the description of the various embodiments of the present invention, a number of terms used herein will be defined.

"Test sample" refers to a material suspected of containing the analyte. The test sample can be used directly as obtained from the source or after pretreatment so as to modify its character. The test sample is typically a physiological fluid. The test sample can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, extracting analyte, or the like. Methods of treatment can involve filtration, distillation, concentration, inactivation of interfering components, and the addition of reagents. Besides physiological fluids, other liquid samples can be used such as water, food products and the like for the performance of environmental or food production assays as well as diagnostic assays. In addition, a solid material suspected of containing the analyte can be used as the test sample once it is modified to form a liquid medium or to release the analyte.

"Specific binding member" refers to a member of a specific binding pair, i.e., two different molecules wherein one of the molecules specifically binds to the second molecule through chemical or physical means. In addition to antigen and antibody specific binding pair members, other exemplary specific binding pairs include, without limitation, such materials as biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, polymeric acids and bases, dyes and protein binders, peptides and specific protein binders (e.g., ribonuclease, S-peptide and ribonuclease S-protein). Furthermore, specific binding pairs can include members that are analogs of the original specific binding member, for example an analyte-analog or a specific binding member made by recombinant techniques or molecular engineering. If the specific binding member is an immunoreactant it can be, for example, an antibody, antigen, hapten, or complex thereof, and if an antibody is used, it can be a monoclonal or polyclonal antibody, a recombinant protein or antibody, a chimeric antibody, a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well-known to those skilled-in-the-art.

"Analyte" or "analyte of interest" refers to the compound or composition to be detected or measured, which has at least one epitope or binding site. The analyte can be any substance for which there exists a naturally occurring analyte-specific binding member or for which an analyte-specific binding member can be prepared.



Analytes include, but are not limited to toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), and metabolites of or antibodies to any of the  
5 above substances. The term "analyte" also includes any antigenic substances, haptens, antibodies, macromolecules and combinations thereof.

"Analyte-analog" refers to a substance which cross-reacts with the analyte-specific binding member, although it may do so to a greater or a lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a  
10 fragmented or synthetic portion of the analyte molecule, so long as the analyte-analog has at least one epitopic site in common with the analyte of interest. An example of an analyte-analog is a synthetic peptide sequence which duplicates at least one epitope of the whole-molecule analyte so that the analyte-analog can bind to an analyte-specific binding member.

15 "Labeled reagent" refers to a substance comprising a detectable label attached to a specific binding member. The attachment may be covalent or non-covalent binding, direct or indirect, but the method of attachment is not critical to the present invention. The label allows the labeled reagent to produce a detectable signal that is directly or inversely related to the amount of analyte in the test sample. The specific  
20 binding member component of the labeled reagent may be selected to directly bind to the analyte or to indirectly bind the analyte by means of an ancillary specific binding member, which is described in greater detail hereinafter. Alternatively, the specific binding member component may be selected to directly or indirectly bind an immobilized reagent. The labeled reagent can be incorporated into the test device, it  
25 can be combined with the test sample to form a test solution, it can be added to the device separately from the test sample or it can be predeposited or reversibly immobilized at the immobilized reagent site. In addition, the binding member may be labeled before or during the performance of the assay by means of a suitable attachment method.

30 "Label" refers to any substance which is capable of producing a signal that is detectable by visual or instrumental means. Various labels suitable for use in the present invention include labels which produce signals through either chemical or physical means. Such labels can include enzymes; enzyme substrates; chromogens; catalysts; fluorescent compounds; chemiluminescent compounds; radioactive labels;  
35 and direct visual labels including colloidal metallic particles such as gold, colloidal non-metallic particles such as selenium, dyed or colored particles such as a dyed

plastic or a stained microorganism, organic polymer latex particles and liposomes or other vesicles containing directly visible substances. A visually detectable label is advantageously used as the label component of the labeled reagent, thereby providing for the direct visual or instrumental readout of the presence or amount of the analyte in the test sample without the need for additional signal producing components at the detection sites.

The selection of a particular label is not critical to the present invention, but the label will be capable of generating a detectable signal either by itself, such as a visually detectable colored organic polymer latex particle, or be instrumentally detectable, such as a fluorescent compound. The label may be detected in conjunction with one or more additional signal producing components, such as an enzyme/substrate signal producing system. A variety of different labeled reagents can be formed by varying either the label component or the specific binding member component of the labeled reagent; it will be appreciated by one skilled-in-the-art that the choice involves consideration of the analyte to be detected and the desired means of detection.

"Signal producing component" refers to any substance capable of reacting with another assay reagent or with the analyte to produce a reaction product or signal that indicates the presence of the analyte and that is detectable by visual or instrumental means. "Signal production system", as used herein, refers to the group of assay reagents that are needed to produce the desired reaction product or signal. One or more signal producing components can be reacted with the label to generate a detectable signal. For example, when the label is an enzyme, amplification of the detectable signal is obtained by reacting the enzyme with one or more substrates or additional enzymes and substrates to produce a detectable reaction product.

"Porous support" refers to any suitable porous, absorbent, bibulous, isotropic or capillary material, which includes the reaction site of the device. Natural, synthetic, or naturally occurring materials that are synthetically modified, can be used as the porous support and include, but are not limited to: papers (fibrous) or membranes (microporous) of cellulose materials such as paper, cellulose, and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; porous fibrous matrixes; starch based materials, such as cross-linked dextran chains; ceramic materials; olefin or thermoplastic materials including films of polyvinyl chloride, polyethylene, polyvinyl acetate, polyamide, polycarbonate, polystyrene, copolymers

of vinyl acetate and vinyl chloride and combinations of polyvinyl chloride-silica; and the like. The porous material should not interfere with the production of a detectable signal.

"Immobilized reagent" refers to a specific binding member that is attached  
5 within or upon a portion of the porous support to form a "capture site" or reactive membrane. The method of attachment is not critical to the present invention. The extent of signal production in the capture site is related to the amount of analyte in the test sample. The immobilized reagent is selected to bind the analyte, the labeled reagent or a complex thereof. In preferred embodiments, the immobilized reagent  
10 binds to the analyte for the completion of a sandwich complex. Competitive assay formats will also be apparent to those skilled-in-the-art. The immobilized reagent may be chosen to directly bind the analyte or indirectly bind the analyte by means of an ancillary specific binding member which itself is bound to the analyte. In addition, the immobilized reagent may be directly or indirectly immobilized on the  
15 solid phase before or during the performance of the assay by means of any suitable attachment method.

Typically, the capture site of the present invention is a delimited or defined portion of the porous support such that the specific binding reaction of the immobilized reagent and analyte is localized or concentrated in a delimited site. Such  
20 a localization facilitates the detection of label that is immobilized at the capture site in contrast to other portions of the porous support. The delimited site is typically less than 50% of the porous support, and preferably less than 25% of the porous support. The immobilized reagent can be applied to the solid phase material by dipping, inscribing with a pen, dispensing through a capillary tube or through the  
25 use of reagent jet-printing or any other suitable dispensing techniques. In addition, the capture site can be marked, for example with a dye, such that the position of the capture site upon the porous support can be visually or instrumentally determined even when there is no label immobilized at the site.

Predetermined amounts of signal producing components and ancillary  
30 reagents can be incorporated within the device, thereby avoiding the need for additional protocol steps or reagent additions. Thus, it is also within the scope of this invention to provide more than one reagent to be immobilized within the porous support. For example, to slow or prevent the diffusion of the detectable reaction product in an enzyme/substrate signal producing system, the substrate can be  
35 immobilized by direct attachment to the porous support by methods well-known in

the art, or the substrate may be immobilized by being covalently bound to insoluble microparticles which have been deposited in and/or on the porous support.

The immobilized reagent may be provided in a single capture or detection site or in multiple sites on or in the porous support. The immobilized reagent may also  
5 be provided in a variety of configurations to produce different detection or measurement formats. Alternatively, the immobilized reagent can be distributed over a large portion of the porous support in a substantially uniform manner to form the capture site.

"Ancillary specific binding member" refers to any member of a specific  
10 binding pair which is used in the assay in addition to the specific binding members of the labeled reagent or immobilized reagent. One or more ancillary specific binding members can be used in an assay. For example, an ancillary specific binding member may be used to bind the labeled reagent to the analyte in instances where the analyte itself could not directly bind the labeled reagent. The ancillary specific  
15 binding member can be incorporated into the assay device or it can be added to the device as a separate reagent solution.

Figure 1 depicts a device wherein the capillary track outlet is in direct contact with the porous support and is positioned beneath the reaction site of  
20 immobilized specific binding material. It is not essential to the present invention that the outlet and porous support be in direct contact. It is sufficient that the components are close enough that fluid in the capillary track will pass from the outlet to the porous support. Nor is it essential that the outlet be positioned directly under the reaction site. It will be readily appreciated, however, that the closer the  
25 capillary track outlet is to the reaction site, the less distance the test sample and/or assay reagents must travel.

Assay reagents, such as a labeled specific binding member, may be mixed with the test sample, may be sequentially contacted to the test device, or may be included within the capillary track. For example, the labeled reagent may be  
30 predeposited in the capillary track such that contact with the test sample mobilizes the labeled reagent and transports the labeled reagent to the reaction site on the porous support. This is to be distinguished from agglutination assay devices wherein the reaction of assay reagents and test sample analyte results in the formation of a reaction product which agglutinates and decreases or stops fluid flow within a  
35 capillary space.

A second embodiment of the present invention is shown in Figure 2. The device (10) generally comprises a main body (12) constructed from a first surface (6) and a parallel second surface (8) one or both of which are grooved, separated by spacers or otherwise constructed such that when the two surfaces are aligned and joined a capillary track (18) is formed. The capillary track has a size and dimensions suitable for the transport of test sample and soluble assay reagents through the track by capillary action. The surfaces may be joined by any suitable means including, but not limited to, sonic welding, solvent welding and adhesive bonding. In the adhesive bonding method, the adhesive may be applied by a printing means.

Figure 3 represents a further embodiment of the present invention. This embodiment has, in adherent relationship, a first or bottom wettable, but liquid-occlusive, layer (22), a second or middle liquid occlusive layer (24) parallel to and overlying the first layer (22), and a third or top liquid-occlusive, preferably non-wettable, layer (26) parallel to and overlying the second layer (24). The third layer (26) may be made from a clear material, such as a clear polycarbonate film, and therefore, the layer may also serve as a window, or viewing area, for observing the capillary track. The second layer (24) is interposed between, and is adhered to, the first layer (22) and third layer (26). For example, the layers are adhered by means of an adhesive on each side of the second layer (24) facing the topside of the first layer (22) and the underside of the third layer (26). Typically, the second layer (24) is die cut or preformed to have a slot positioned through its thickness, thereby defining the walls of the capillary track (18) in conjunction with the first (22) and third (26) layers. Thus, when the first, second and third layers are laminated together, a portion of each of the first and third layers serve as the floor and roof, respectively, of the capillary track with part of the walls of the slot of the second layer (24) defining the walls of the capillary track.

The device illustrated in Figure 3 may also include an optional well-defining means (2) in the third layer (26). The well-defining means is positioned such that it defines an area for receiving the test sample, and it is in fluid flow communication with the capillary track. The bottom of the well may be formed from a corresponding circular portion of the first layer (22).

In a preferred embodiment, the devices of the present invention include a test sample application pad in fluid flow contact with the capillary track. The application pad facilitates the application of test sample or reagents to the device and may optionally contain one or more reagents, such as the labeled binding member. The

addition of test sample to the application pad serves to elute an assay reagent from the application pad, such that a test sample/reagent mixture emerges from the bottom surface of the application pad. The device may further include a well situated between the application pad and the capillary track inlet such that the test solution exiting the application pad substantially fills the well prior to passing into the capillary track. The application pad may be constructed from a single material or from a plurality of layers. The use of a multi-layered application pad permits the inclusion of multiple assay reagents, even when the reagents are not compatible for extended storage, thereby allowing multiple, separate reagent additions to the test sample. The application pad material or a layer thereof may also be selected to provide a filter function.

Figure 3a depicts an alternative embodiment wherein the device includes a drop-forming means (50) which holds an application pad (55) that contains the labeled reagent. By contacting a test sample to the drop-forming means, the labeled reagent is released from the application pad and forms a drop on the bottom surface of the application pad. The drop-forming means is situated over the capillary track such that when the drop is released from the application pad it is delivered to the capillary track. This optional modification provides an added advantage. The addition of fluid or test sample to the reagent-containing application pad serves to deliver a bolus of the eluted reagent to the capillary track. Thus, the first fluid mixture entering the capillary track contains a large portion of the eluted reagent, and subsequent fluid contains less of the reagent. When the reagent is a labeled reagent, this means that the first fluid delivered to the reaction site on the porous support contains the largest portion of the labeled reagent. Subsequent fluid contains a lesser amount of assay reagent and thereby enhances the clearance of unreacted reagents from the reaction site. This clearance or washing aspect of the invention helps to stabilize the signal that is produced in the reaction site and decreases interference from the occurrence of background signal in the area surrounding the reaction site.

In yet another embodiment, as depicted in Figure 4, the sides (24) of the capillary track (18) are formed from a printable material that is insoluble, and preferably, that aids in adhering the first liquid occlusive layer (22) to a third liquid occlusive layer (26). The material is applied to either the first or third layer, and the sides are then capped by the application of the third or first laminate layer, respectively, thereby defining the capillary track (18).

The first (back laminate) layer may be a flexible plastic or plastic-coated film supplied in either sheet or roll form. The film can also be chosen to have

distinct properties such as opacity, biodegradability, etc., or it can be treated to have certain properties, such as hydrophobicity, hydrophilicity or selective biocompatibility. The film may be selected from a variety of materials including, but not limited to, polyester, polycarbonate, and other film materials. In addition, the surface or a portion of the surface of the capillary track may be spot treated to create or enhance a desired property. For example, a portion of the capillary track may be spot treated with a hydrophobic material to slow the rate of flow through that portion of the track. Suitable film materials include, but are not limited to, Mylar® film (DuPont, Wilmington, DE) and polyester films (Melinex® films; ICI Films, Wilmington, DE). Suitable film materials include materials having the following properties or characteristics:

<u>Film Properties</u>	<u>Typical</u>	<u>Range</u>
Thickness:	0.002 inch	0.0002 - 6.0 inches
Flexible:	30,500 psi tensile	any flexible or rigid material
Optical:	opaque white	transparent, opaque, reflective, metallic, or treated
Material:	polyester	any plastic, glass or metal or combination thereof

The second (core or sandwiched) layer is conveniently applied via printing techniques, such as screen printing methods which are well-known in the art. The second layer may be applied or deposited, however, by any suitable printing method capable of achieving the desired design tolerances for thickness, alignment, or geometric limitations of the capillary track. It will be appreciated by those skilled-in-the-art that the capillary track dimensions will be selected to accomplish the desired fluid delivery and timing characteristics which may differ between devices based upon the analyte of interest, the test sample used, incubation and reaction requirements, and other assay parameters. The second layer may be printed from an adhesive material, an ink, a dielectric material, or any material that is suitable for printing and for providing the desired thickness or height of the capillary track. The second layer may preferably be formed from an adhesive. More preferably from a pressure sensitive adhesive. Pressure sensitive adhesive materials generally consist of a polymer formulation and are usually vinyl or acrylic based (e.g., UVC

8201, UVC 8200, ML 25184; Acheson Colloids, Port Huron, MI). Suitable pressure sensitive adhesives include, but are not limited to, materials having properties similar to UVC 8201 polyester film:

<u>Adhesive Properties</u>	<u>Typical</u>	<u>Range</u>
Solids Content:	100% solids	20% - 100%
Density:	8.48 lbs./gal.	5.4 - 13.4 lbs./gal.
Viscosity:	1700 - 2000 cps.	100 - 750,000 cps.
Color:	transparent	any
Cure:	U.V.	air dry, heat cure, U.V., solvent evaporation, crosslink
Material:	Urethane Acrylate	any -- acrylic, epoxy, vinyl, conductive, non- conductive, adhesive
Coverage:	1600 sq. ft./gal @ 1 mil	any -- to meet the desired film thickness
Adhesion (90 degree peel test):	2 lbs./in.	0.25 - 100 lbs./in.
Printed Film Thickness:	1 - 5 mils	2 - 10 mils

5

The printed material may be selected to have a suitable adhesiveness to laminate the top and bottom layers, as well as a suitable hydrophilic characteristic to promote the movement of fluid through the capillary track. Alternatively, the printed material may be selected to have a suitable hydrophobic characteristic to inhibit or prevent fluid flow within a portion of the capillary track, thereby controlling the rate of fluid flow through the device.

10

The pressure sensitive adhesive is applied in the reverse image of the capillary track, such that the printed area or areas define the thickness of the core layer and the non-printed area or areas form a gap between the first and second laminate layers, thereby defining the sides of the capillary track. The pressure sensitive adhesive may be applied using standard screen printing equipment (such as a flatbed screen printer from deHaart Inc, Burlington, MA). Typically, the second layer is applied in a single printing pass to a thickness ranging from 0.0002 inches to 0.010 inches. The layer, however, can be formed to have any desired thickness if

15



accomplished in multiple passes. Usually, the layer will not exceed a final thickness of 0.100 inches.

Following application, the pressure sensitive adhesive is cured. For example, the pressure sensitive adhesive may be cured by means of ultraviolet radiation at about 200 watts per inch, or any other power rating that accomplishes crosslinking of the polymer to achieve the desired film properties such as thickness, adhesion and tack. A release liner may be placed over the printed adhesive for handling purposes until device assembly, although assembly can take place immediately after cure. In conventional device construction methods involving a die cut film material with a two-sided adhesive, there are two layers of release liner to be removed prior to assembly, and assembly is more cumbersome. In the present invention, the core layer of printed pressure sensitive adhesive is applied directly to one side of the base or back film, thereby avoiding this handling step.

The third (base) layer may be selected from the same film material as the first laminate layer. A different material may be used, however, to meet desired fluid flow characteristics or other properties pertinent to the design of the desired capillary track. A plastic or plastic-coated film, chosen for properties of opacity and wettability (e.g., plastic-coated paper board 150HT, Daubert, Dixon, IL; Vistex PC polyester film, Film Specialties, Inc., Whitehouse, NJ), is received in a roll and is applied to the printed core layer via standard web-laminating or processing techniques which are well-known in the art. Pressure is applied to bond the back and base layers to the core of pressure sensitive adhesive thereby forming the top and bottom of the capillary track. The base film may be slit, or die punched, or laser cut to produce fixture holes or other features of the desired design such as inlet and outlet ports for the capillary track. The third film layer, like the first, can be treated to have certain properties, such as hydrophobicity, hydrophilicity or selective biocompatibility. Either the whole layer may be treated or at least that portion of the layer which forms the capillary track may be treated. Such treatment materials may also be advantageously applied by printing techniques.

In yet another embodiment, as depicted in Figure 5, the second layer (24) is made of a porous or liquid absorbent material which is selectively impregnated through its thickness with a substance, such as a water-repellent ink, to form an impregnated region (30) and a non-impregnated region (18). The core layer of porous material defines the thickness of the gap between the top and bottom laminate layers, and the impregnated regions of the porous material define the side walls of the capillary track (18). The non-impregnated region remains liquid absorbent,

and the impregnated region is made liquid-occlusive, such that the non-impregnated region defines a solid capillary track for the passage of fluid via capillary action.

Thus, the non-impregnated region, with inlet and outlet portions, serves as the means for directing the test solution through the device to the overlying porous material.

The porous second or core layer can be constructed using any suitable porous medium which typically has characteristics similar to the porous support materials. An exemplary porous medium is conventional filter paper (Whatman, UK; or Schleicher & Schuell 410, Keene, NH). The porous medium is generally printed with a pressure sensitive adhesive or ink by means of equipment and printing techniques well-known in the art. The printed pattern defines the capillary track because the pressure sensitive adhesive or ink inhibits fluid-flow through the printed portions of the porous medium. The use of a pressure sensitive adhesive to print the porous medium has the added advantage of providing the adhesiveness for applying the base or back laminate layers. The conventional method of device assembly involved cutting strips or pieces of the porous media and sandwiching that media between the back and base layers. With the present method, the roll of printed porous material is simply incorporated into the web process, thereby eliminating costly and complicated pick and place operations. Printable inks may be formulated to contain an assay reagent which is released from the printed layer as the test sample passes over the printed material. The filtering capacity of the porous medium in the capillary track may advantageously be used in the assay protocol. Moreover, different portions of the porous medium in the capillary track may be treated to modify the filter or transport characteristics of the medium. In addition, different portions of the porous medium in the capillary track may be treated to contain one or more assay reagent zones from which a reagent is released upon contacting the transported fluid.

The use of a printable medium to define the capillary track also provides for limitless design opportunities in terms of the geometric shape and variable thicknesses of the capillary track which characteristics may be used to control the rate at which the assay is performed. In addition, it simplifies device manufacture by eliminating the need for additional layer materials and material handling, while enhancing batch manufacturing procedures. The application of pressure sensitive adhesives and ink materials can be accomplished with any suitable method, including but not limited to, rotary or flatbed screen printing, flexographic printing, lithographic printing, letterpress printing, rotogravure printing, or ink jet

printing. The devices may be printed in either a batch mode (one sheet at a time on either flexible or rigid film material) or in a roll with web processing methods (on primarily flexible film material). The printing processes can be accomplished with standard equipment. Exemplary processes are described in "The Printing Ink  
5 Manual" by R.H. Leach; "Handbook of Thick Film Technology" by P.J. Holmes and R. G. Loasby; or "Handbook of Thick Film Microcircuits" by Charles A Harper. Set up of the printer is within standard parameters and processes known in the art, or as described in the relevant instruction manual or in the above-mentioned texts. Typical ink properties are also described, and for purposes of the present invention,  
10 the characteristics of suitable inks are similar to the characteristics of the printable pressure sensitive adhesives.

In yet another embodiment, as depicted in Figure 6, the sides of the capillary track are defined by a solid middle or core layer (24) of film material which is laminated between a first layer (22) and a third layer (26) using two layers of a  
15 suitable adhesive material (28). The middle layer has a slot within its surface, partially or completely through the material, such that in combination with the first and/or third layers a capillary track (18) is formed. The adhesive material may also be die cut or printed to complement the slot in the middle layer such that the adhesive material does not form a part of the capillary track.

20 In an optional modification of the device, the area of the porous support around the immobilized reagent may be at least partially compressed. Figure 7 depicts an embodiment wherein a nitrocellulose material (20) has been compressed in the area around the immobilized reagent site (22) to aid in the direction of fluid flow through the porous support and reaction site.

25 In another embodiment, the device may include an additional absorbent material in contact with the porous support. Figure 8 illustrates an embodiment representing a device which includes an absorbent layer (30) surrounding the reaction site (22) on the porous support (20). The absorbent material serves to increase the liquid holding capacity of the device such that large test sample or  
30 reagent volumes may be used.

The main body of the devices of the present invention may be formed of a non-wettable material, such as a plastic material, or a wettable material, such as a porous material, wherein at least a portion has been rendered non-wettable. The  
35 capillary track typically has a total length of from about 0.5 to about 6.0 inches, preferably from about 0.5 to about 2.0 inches. The structural arrangement of the

device is generally designed such that about 50 to about 1000 microliters of test sample may be used to perform an assay. Preferably, the device is designed such that about 100 to 500 microliters of test sample is used. It will be appreciated by those skilled-in-the-art that the design will be optimized as needed to provide for the use of that amount of test sample required to perform the desired assay. The capillary track has a diameter suitable for the transport of such samples from the capillary track inlet to the capillary track outlet.

The test sample is introduced to the reaction zone in the porous support by means of the subsurface capillary track. The subsurface capillary track reduces the time required for a unit volume application of test sample to wet the same surface area of the porous support. Because the test sample and assay reagents are delivered directly to the reaction site, the test sample is not required to first pass through a non-reaction site portion of the porous support. This advantageous outcome is described by the D'Arcy Equation.

The D'Arcy Equation expresses flow rate as follows:

$$h^2 = kt$$

where  $h$  = distance of chromatographic flow

$k$  = flow constant

$t$  = time

Where  $A_1$  is equal to the area of a porous material in the shape of an elongated strip ( $A_1 = h_1 \times w_1$ ), and  $A_2$  is equal to the area of the porous support in the shape of a square or circle ( $A_2 = h_2^2 \pi$ ) of the same thickness, the unit volume of test sample applied to each device format will flow as depicted in Figure 9. The linear uptake of a unit volume is substantially slower than that of radial uptake.

The present invention also provides for the ability to simultaneously perform multiple assays while utilizing a very small amount of test sample, for instance, a single drop. Such a device, in assembled form, has a plurality of capillary tracks. The device includes a sample application means which communicates with the inlet of the capillary tracks. The outlet of each track is in communication with a porous support. The individual porous supports are selectively impregnated with a specific binding member suitable for the detection of an analyte of interest. The number of tracks is not critical to the construction of the multiple track devices of the present invention. Alternatively, a single capillary track can include multiple outlets such that different outlets underlie different portions of a single porous support, wherein the different portions of that porous support contain immobilized binding members for different assays.

The assay devices of the present invention include a porous support in liquid communication with a capillary track, which support is typically positioned adjacent to, and usually in direct contact with, the capillary track outlet. However, it has been discovered that the devices can include additional zones or layers between the capillary track and the porous support. Such zones may be used to further control the rate of flow between the capillary track and the porous support, may contain ancillary assay reagents or may be used to prevent or inhibit the transport of test sample interferences into the porous support.

Optionally, the flow rate per unit area of the capillary track can be gradually decreased along the general direction of flow by gradually increasing the space between the floor and the roof of the track along the direction of liquid flow. For example, flow may be decreased by gradually bowing the roof of the track upward and/or gradually bowing the floor of the track downward.

In an alternative embodiment of the present invention, a labeled reagent is positioned in the interior of the capillary track to form a reagent zone. When a test sample is introduced to one end of a capillary track, the test sample and labeled reagent combine to form a test solution. The test solution is transported through the track. The rate of liquid flow through the capillary track may be controlled, at least in part, by means of the porous support positioned at the distal end of the track. It has been found that a porous material, such as paper, may be utilized as the fluid flow control means to provide advantages in both manufacturing and performance over the coating of the track interior with water-soluble materials such as polyvinylpyrrolidone (PVP).

All types of specific binding assays can be accommodated with a device constructed in accordance with the present invention. Moreover, with the inclusion of all necessary assay reagents within the device itself, the assays may be made essentially self-performing once the test sample has been added to the device. For example, a soluble reagent (such as a labeled reagent) is dried within the capillary track during manufacture and is solubilized upon contact with the test sample. In other instances, a labeled reagent can be contained by a soluble or porous matrix which is positioned within the capillary track itself. Suitable matrices which can release diffusive materials are well known in the art and include, but are not limited to, paper, sponge and glass fiber materials. In yet another embodiment, a reagent can be dispersed in a solution which is placed in the track.

The very small size of the reaction devices of the present invention advantageously allows for the rapid and convenient handling of a plurality of devices.

A device can then be loaded into an automated apparatus which indexes and scans the individual reaction sites for the assay results and records this information for future access. The small dimensions of the device also provide for efficient use of sample and reagents. The present invention also provides for diagnostic kits  
5 employing the present devices in combination with containers of assay reagents which are not incorporated within the device itself, for example, a test sample buffer solution or extraction solution.

## EXAMPLES

10

The following examples are provided to further illustrate embodiments of the invention and should not be construed as a limitation on the scope of the invention.

### EXAMPLE 1

15

A disposable device, as depicted in Figure 3, was constructed from a wettable base layer (22) (7 mil, hydromer-treated polyester; Film-Specialties, Inc., Whitehouse, NJ), a die-cut adhesive core layer (24) (3 mil, double sided adhesive coated polyester film; Adhesives Research, Glen Rock, PA), a laser-machined, non-  
20 wettable, adhesive, laminate layer (26) (3 mil, single sided adhesive coated polyester film, Adhesive Research) and a microporous nitrocellulose pad (20) (5 micron pore size; Schleicher & Schuell, Keene, NH). The assembly of the wettable base, die cut core layer and laser-machined laminate form a subsurface capillary track with an inlet (2) at one end and a small outlet (16) at the other. The  
25 nitrocellulose membrane is laminated over the outlet to allow sample to dispense from the capillary track (18) into the membrane center thus avoiding linear chromatography from the membrane edge.

### EXAMPLE 2

30

#### Human Chorionic Gonadotropin (hCG) Assay

A disposable device was constructed substantially in accordance with the description of Example 1. Anti-beta hCG antibody was applied to the center of the nitrocellulose pad in a "+" pattern with the two bars intersecting over the outlet in  
35 the laser-machined laminate. One of the bars included hCG to serve as a positive control for hCG-negative samples. Anti-alpha hCG antibody, and protein stabilizers,

were absorbed on selenium particles (180 nm) to provide the labeled reagent for this sandwich assay.

Dry selenium conjugate pads were prepared by dipping glass fiber strips (Lydall, Inc., Rochester, NH) into a selenium conjugate solution and then passing the material through a drying tunnel. Circles (approximately 0.023 inches in diameter) were punched from the material and held in a molded drop forming means as shown in Figure 3a.

An hCG test sample (250 to 400 microliters) containing buffer or urine was applied to the assembly. A bolus of selenium conjugate was delivered to the track if the drop forming means was held above the track to allow a hanging drop to form. The drop fell onto the capillary inlet, filled the capillary track and was transported to the nitrocellulose pad and through the capillary outlet directly beneath the center of the immobilized reagent "+". As the solution was radially transported in 360 degrees through and from the immobilized reagent site, a visible signal was formed at the reaction site in the form of a "+" if hCG was present in the sample, and in the form of a "-" if no hCG was present.

#### Strep A Assay

A disposable device was constructed substantially in accordance with the description of Example 1. Anti-Strep A antibody was applied to the center of the nitrocellulose pad in a "+" pattern with the two bars intersecting over the uptake hole in the laser-machined laminate. One of the bars included a protein containing the immunodeterminant recognized by the anti-Strep A antibody and served as a positive control for Strep A-negative samples. Anti-Strep A polyclonal antibodies, with protein stabilizers, were absorbed on selenium particles (180 nm) which served as the labeled reagent for this sandwich assay.

Dry selenium conjugate pads and drop forming means were constructed as described above. Upon application of the test sample, a signal developed at the reaction site in the form of a "+" if Strep A immunodeterminant was present in the sample, and in the form of a "-" if no Strep A immunodeterminant was present.

#### Results

Upon applying a test sample to the devices of the present invention, the average time for the sample to contact the nitrocellulose pad was five to ten seconds. Depending on the test sample hCG concentration, signal could be seen as a "+" in 30-40 seconds (250 mIU/ml) or between one and three minutes for test sample

concentrations of 5-25 mIU/ml. Similar results were found for high and low levels of Strep A immunodeterminate in a test sample. It will be appreciated by those skilled-in-the-art that these reaction times may be further modified through reagent optimization. A novel and unexpected aspect of this technology is that the labeled reagent passing from the conjugate pad is concentrated in the first drop dispensed from the drop forming means into the capillary track. Typically, over 50% of the conjugate is concentrated in the first formed drop. Preferably, over 70% of the conjugate is concentrated in the first formed drop. In the most preferred form, over 90% of the conjugate is concentrated in the first formed drop. The immobilized reagent is then subjected to a bolus delivery of labeled reagent with the test sample, and as subsequent test sample passes through the test device unreacted labeled reagent is cleared from the reaction zone. The resulting signal was thus enhanced as the background field of the immobilized reaction site changed from pink to white and the immobilized signal remained red.

15

### EXAMPLE 3

A disposable device was constructed from a wettable base (7 mil hydromer-treated polyester, Hydromer, Inc.), a die-cut adhesive core layer (3 mil, double sided adhesive coated polyester film (Adhesives Research) and a printed filter-paper top layer (S&S grade 410). A hole was punched in the printed part of the filter paper layer to serve as a sample entry means. The assembly of the wettable base, die-cut core layer and printed filter-paper top layer formed a subsurface capillary track with a sample uptake hole in the paper top layer where ink was not deposited. The printed pattern in the track allowed sample to fill the track to a predetermined point. The printed pattern in the top layer allowed solution to travel within the track and react with reagents that were deposited in the wettable, non-printed areas of the top paper layer.

30

### EXAMPLE 4

#### Glucose Test

A glucose test device was constructed by using a disposable device as described in Example 3 and assay reagents for an enzymatic glucose determination. The color forming reagent was 4-chloronaphthol which was spotted onto the filter paper as 25 mg/ml acetone solution and allowed to dry. Solutions of glucose oxidase and

35



horseradish peroxidase (Sigma, Inc.; St. Louis, MO) in a phosphate buffer were then spotted on the filter paper in the wettable regions containing the dried 4-chloronaphthol. Concentrations of glucose oxidase were chosen to allow the color formation reaction to proceed at different rates between each wettable region. The test was begun by adding a buffered solution containing glucose to the entry hole.

### Results

Upon sample application, the fluid flowed via capillary action through all of the wettable regions and color formation began. Rates were monitored using a light transmission device. Each wettable area was monitored independently.

### EXAMPLE 5

A disposable device and reagents, as described in Example 3 and 4, were used to make a glucose test device. The wettable base layer had a pattern of hydrophobic ink printed down the capillary track. Upon application of a buffered solution containing glucose, the track filled down to the hydrophobic ink section of the track. The device was attached to a rotating means (e.g., Dremel tool) through a hole in the center of the device. Upon applying centrifugal force to the device, the sample was forced past the hydrophobic track section and into a holding area of the track. Upon release of the centrifugal force, the solution travelled up the track into the reagent area where the glucose-determining reactions began.

Blood separation was accomplished in the device by applying a whole blood sample to the sample uptake hole as described above for the buffered-glucose solution. Upon application of centrifugal force the blood cells separated out in the bottom of the track past the hydrophobic track area. With the release of the centrifugal force, plasma decanted away from the compacted cells and travelled up into the reagent portion of the test where the glucose-determining reactions began.

### EXAMPLE 6

A disposable device is constructed by using a film material as the first or base layer. The film may also be used as the third or top layer. The top layer is cut by a laser to form two holes which provide the capillary inlet and outlet ports once the device is constructed. The bottom surface of the third layer is screen printed with an aqueous adhesive leaving an unprinted area between the two holes which

defines the sides of the capillary track. The bottom surface of the third layer provides the top of the capillary track. The adhesive material is cured, and the base layer is placed over the printed material. A suitable amount of pressure is applied to adhere the the base layer, thereby forming the bottom of the capillary track.

5 A porous support is positioned on the upper surface of the top layer substantially over the capillary track outlet such that the outlet is in fluid flow communication with the porous support. An assay reagent may be immobilized on the porous support before or after the support is attached to the film.

10

## EXAMPLE 7

A disposable device is constructed substantially in accordance with the technique described in Example 6, with the exception that an adhesive is applied to both the first and third layers.

15

## EXAMPLE 8

A disposable device is constructed substantially in accordance with the technique described in Example 6, with the exception that a two-sided adhesive  
20 material having suitable release liners and a slot to define the sides of the capillary track is applied to either the first or third layer to form a middle layer. For example, a release liner is removed from one side of the adhesive material and aligned over the base layer, and pressure is applied. The second release liner is then removed, the third layer is aligned over the adhesive, and pressure is applied to  
25 complete the construction of the capillary track.

It will be appreciated by those skilled-in-the-art that the concepts of the present invention are applicable to various types of assay configurations, analytes,  
30 labels and device materials. The embodiments described herein are intended as examples, rather than as limitations, of assay devices using subsurface flow. Thus, the description of the invention is not intended to limit the invention to the particular embodiments disclosed, but it is intended to encompass all equivalents and subject matter within the scope of the invention as described above and as set forth  
35 in the following claims.

## CLAIMS

What is claimed is::

- 5     1.     A device for performing a specific binding assay to determine the presence or amount of an analyte in a test sample, comprising:  
          a capillary track having an inlet and outlet, wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test sample or test solution to;
- 10           a porous support containing an immobilized reagent which binds a member selected from the group consisting of the analyte, an ancillary specific binding member and a labeled reagent, wherein said outlet port is disposed beneath said immobilized reagent in said porous support.
- 15     2.     The device according to Claim 1, wherein at least a portion of said porous support is compressed in the area surrounding said reagent, wherein the compressed area directs the flow of test sample or test solution through said porous support.
3.     The device according to Claim 1, wherein said labeled reagent is contained  
20     within said capillary track.
4.     The device according to Claim 1, wherein said labeled reagent is contained by a reagent matrix within a drop forming means which is in communication with said capillary track.
- 25     5.     The device according to Claim 1, wherein said labeled reagent is contained by a porous reagent matrix within said capillary track.
6.     The device according to Claim 1, further comprising a test sample well  
30     communicating with said capillary track inlet.
7.     The device according to Claim 1, wherein said capillary track outlet directs the test sample to a bottom surface of said porous support directly beneath said immobilized reagent.

8. The device according to Claim 1, wherein said capillary track comprises at least a first and a second capillary track wherein said first capillary track contains a labeled reagent, and wherein said second capillary track directs test sample to said porous support at a faster rate than does said first capillary track, whereby at least  
5 a portion of the analyte is transported to the immobilized reagent prior to said labeled reagent reaching said porous support.

9. The device according to Claim 1, wherein said capillary track is constructed from a first layer and a second layer of liquid impermeable materials having opposed  
10 interior bottom and top surfaces, respectively, which define said capillary track.

10. A method for detecting the presence or amount of analyte in a test sample, comprising the steps of:  
a) contacting the test sample to a capillary track having an inlet and outlet,  
15 wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test sample or test solution to,  
a porous support containing an immobilized reagent which directly or indirectly binds the analyte, wherein said outlet port is disposed beneath said immobilized reagent in said porous support, and wherein a labeled reagent directly  
20 or indirectly binds to the analyte to form a labeled complex on said porous support in relation to the presence or amount of analyte in a test sample; and  
b) detecting said labeled reagent in said porous support to determine the presence or amount of analyte in a test sample.

25 11. A method for detecting the presence or amount of analyte in a test sample, comprising the steps of:  
a) contacting the test sample to a capillary track having an inlet and outlet, wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test sample or test solution to,  
30 a porous support containing an immobilized reagent which directly or indirectly binds the analyte, wherein said outlet port is disposed beneath said immobilized reagent in said porous support, and wherein a labeled reagent competes with the analyte to bind said immobilized reagent thereby forming a labeled complex on said porous support in relation to the presence or amount of analyte in a test  
35 sample; and

b) detecting said labeled reagent in said porous support to determine the presence or amount of analyte in a test sample.

12. A method for detecting the presence or amount of analyte in a test sample, comprising the steps of:

- 5 a) contacting the test sample to a capillary track having an inlet and outlet, wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test sample or test solution to,
- 10 a porous support containing an immobilized reagent which competes with the analyte in directly or indirectly binding to a labeled reagent, wherein said outlet port is disposed beneath said immobilized reagent in said porous support, thereby forming a labeled complex on said porous support in relation to the presence or amount of analyte in a test sample; and
- 15 b) detecting said labeled reagent in said porous support to determine the presence or amount of analyte in a test sample.

13. A kit for performing a specific binding assay to determine the presence or amount of an analyte in a test sample, comprising:

- 20 a) a device involving a capillary track having an inlet and outlet, wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test sample or test solution to;
- a porous support containing an immobilized binding reagent, wherein said outlet port is disposed beneath said immobilized reagent in said porous support; and
- 25 b) instructions for the performance of the assay.

14. The kit according to Claim 13, wherein said device further comprises a test sample application pad containing a labeled reagent, wherein said pad is in fluid flow contact with said capillary track inlet.

30 15. A device for determining the presence or amount of an analyte in a test sample, comprising:

- a capillary track having an inlet and outlet, wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test sample or test solution to a porous support;
- 35 wherein said capillary track is constructed from a first and a second film layer, defining a top and a bottom of said capillary track, and a printed core layer

applied to said first or second layer in a reverse image of said track and having a thickness which defines side walls of said capillary track; and

said porous support containing an immobilized reagent which directly or indirectly binds a labeled reagent in relation to the presence or amount of analyte in the test sample, wherein said outlet port is disposed beneath said immobilized reagent in said porous support.

16. The device according to Claim 15, wherein said printed layer is an adhesive material.

10

17. A method for constructing a capillary track, comprising the steps of:

a) applying a printable material to a first film layer thereby forming a core layer having a thickness which defines the side walls of the capillary track, wherein said printable material is deposited as a reverse image of the capillary track on said

15 first film layer; and

b) adhering a second film layer to said printable material, thereby forming the fourth side of the capillary track.

18. The method according to Claim 17, wherein said printable material is selected from the group consisting of an adhesive, an ink and a dielectric material.

20

19. The method according to Claim 17, wherein said printable material is a pressure sensitive adhesive.

20. The method according to Claim 17, further comprising the step of treating the capillary track to modify the hydrophobicity, hydrophilicity or selective biocompatibility of said film layers.

25

21. A method for constructing a capillary track, comprising the steps of:

30 a) applying a fluid repellant printable substance to a porous material thereby impregnating said porous material wherein a non-impregnated region defines two sides of the capillary track and the thickness of said porous material defines the height of the capillary track; and

b) adhering a first and a second laminate layer to said porous material thereby forming a top and a bottom of the capillary track.

35

22. The method according to Claim 21, wherein said fluid repellant printable substance is an ink.

23. The method according to Claim 21, wherein said fluid repellant printable  
5 substance is an adhesive.

24. The method according to Claim 21, further comprising the step of treating porous material to modify the hydrophobicity, hydrophilicity or selective biocompatibility of the capillary track.

10

25. A device for determining the presence or amount of an analyte in a test sample, comprising:

a capillary track having an inlet and outlet, wherein said inlet receives test sample or test solution, and said outlet is in communication with and directs test  
15 sample or test solution to a porous support;

wherein said capillary track is constructed from a porous material selectively impregnated with a fluid repellant printable substance wherein a non-impregnated region defines two sides of said capillary track, and wherein said porous material forms a core layer between a first and a second laminate layers which form  
20 a top and a bottom of said capillary track; and

said porous support containing an immobilized reagent which directly or indirectly binds a labeled reagent in relation to the presence or amount of analyte in the test sample, wherein said outlet port is disposed beneath said porous support.

25

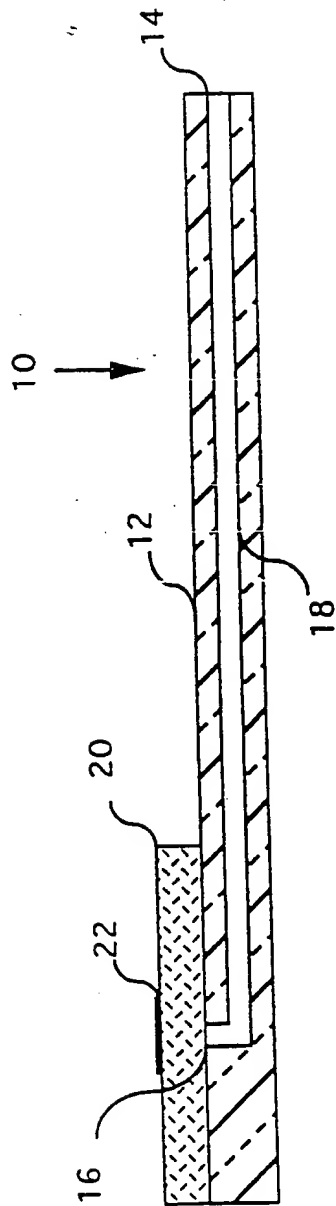


Figure 1

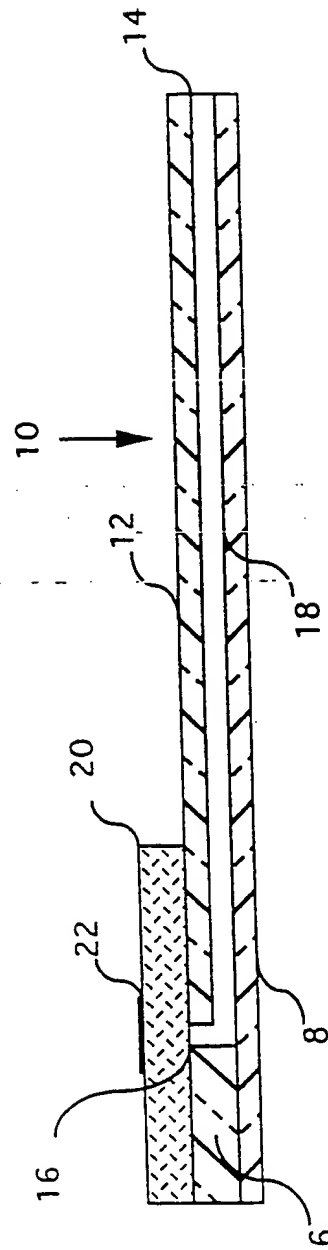


Figure 2



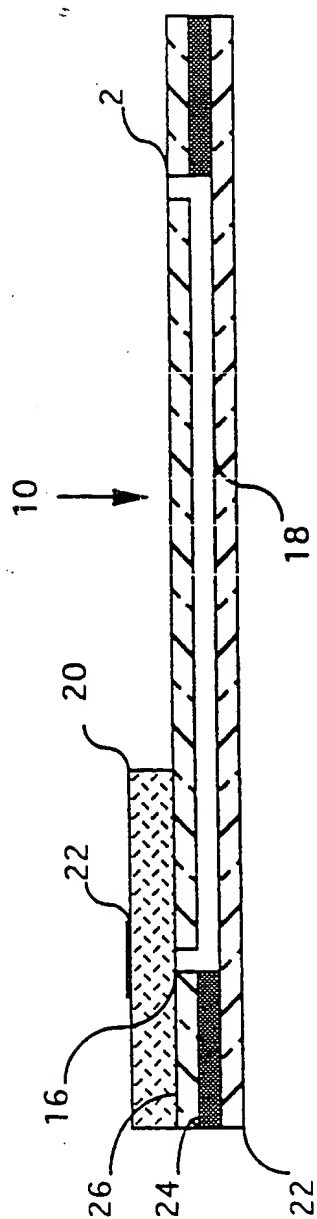


Figure 3

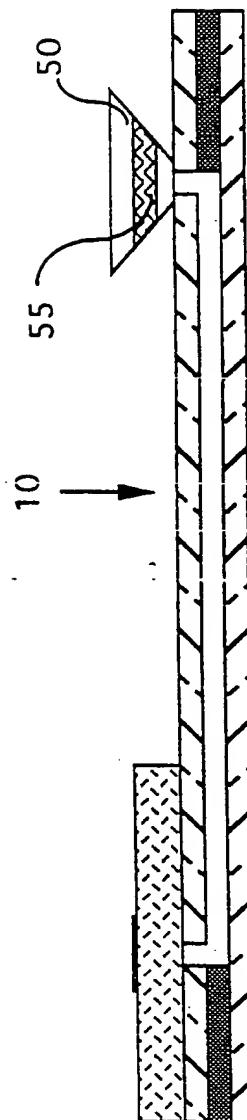


Figure 3a

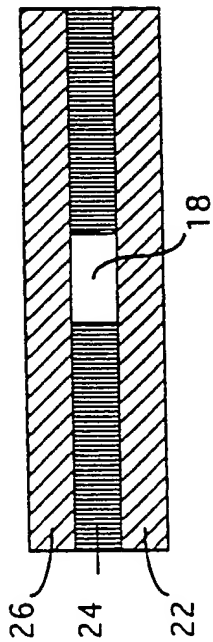


Figure 4

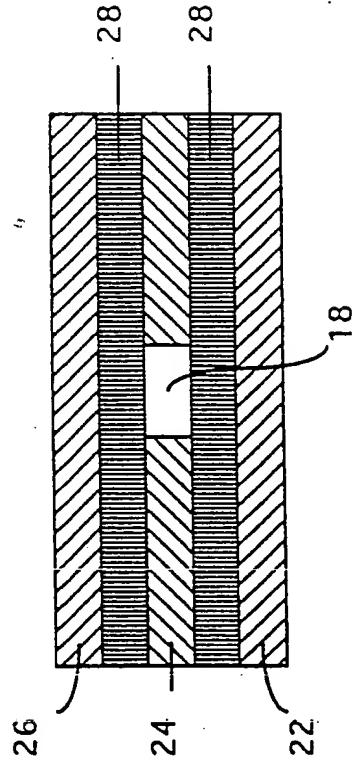


Figure 6

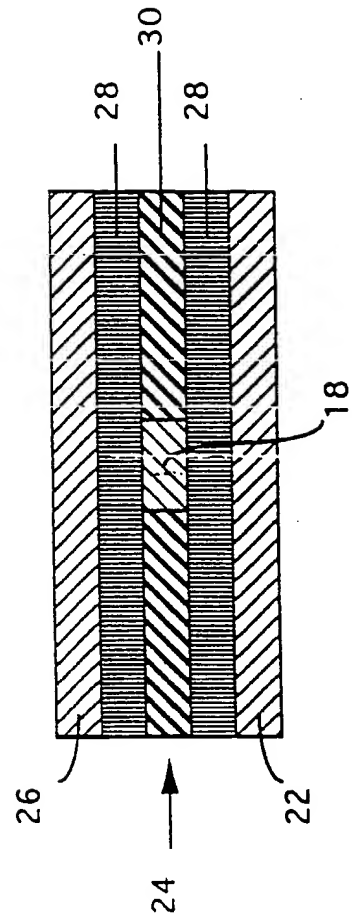


Figure 5

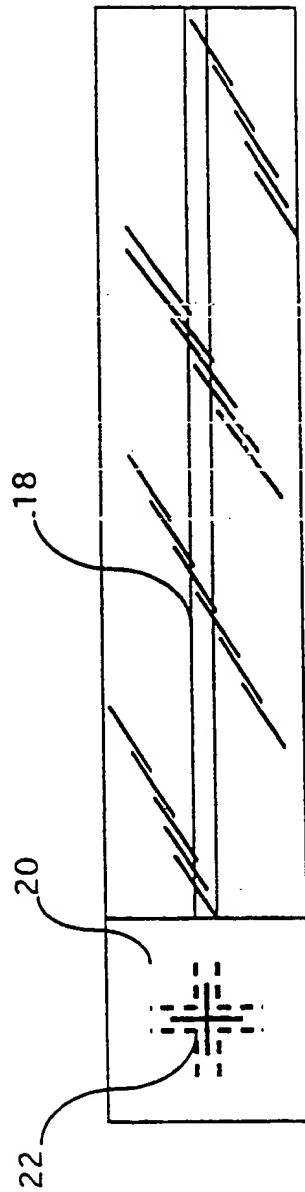


Figure 7

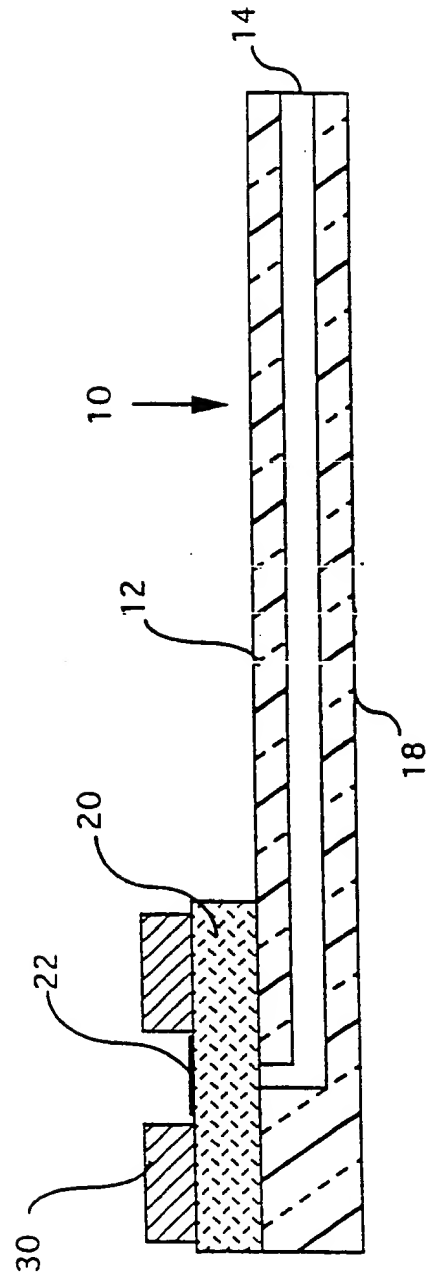


Figure 8

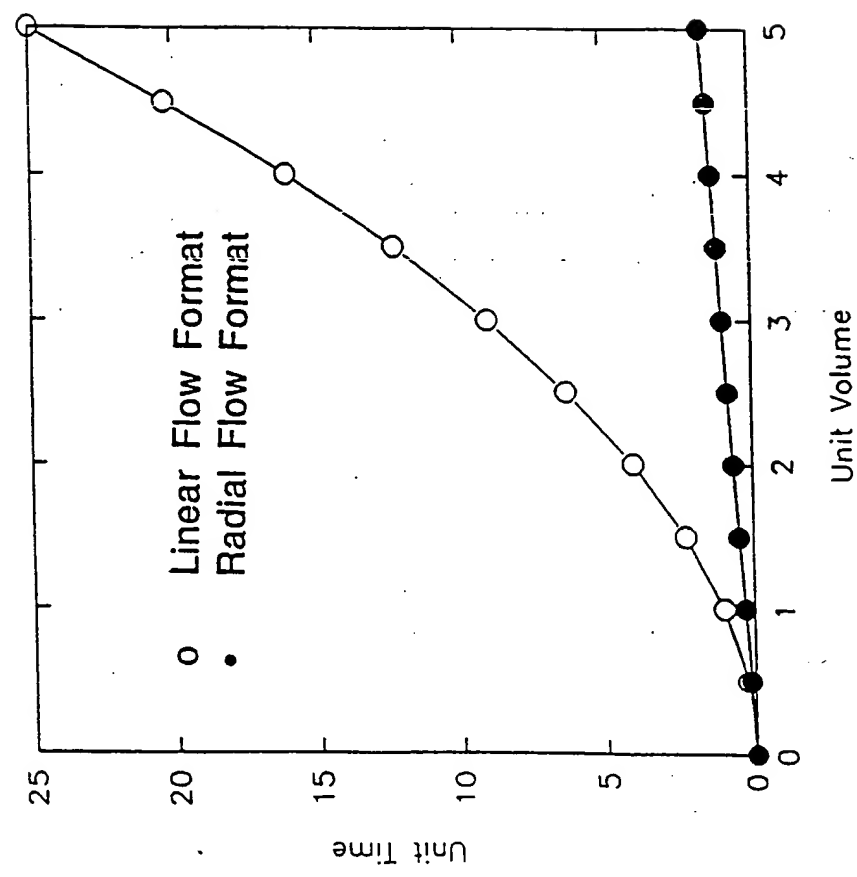


Figure 9

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/08751

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 33/543

US CL :436/518; 422/58

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NoneElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
None

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P ----- Y	US, A, 5,202,268 (Kuhn et al), 13 April 1993, see entire reference.	1-3,5-7,9-12 ----- 13
X	US, A, 4,790,640 (Nason) 13 December 1988, see entire reference.	15-20

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

- \* Special categories of cited documents:
- \*A\* document defining the general state of the art which is not considered to be part of particular relevance
- \*E\* earlier document published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*Z\* document member of the same patent family

Date of the actual completion of the international search

19 October 1993

Date of mailing of the international search report

DEC 02 1993

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

CHRISTOPHER CHIN

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/08751

## B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

156/277,297;

422/55,56,57,58;

427/207.1,208.4,208.6,256;

435/4,7.9,7.92,7.93,7.94,805,970;

436/169,170,514,518,527,528,530,531,538,541,805,807,810;